Visualizing Hypothalamic Network Dynamics for Appetitive and Consummatory Behaviors

Graphical Abstract

Highlights
- Activation of LH GABAergic neurons produces appetitive and consummatory behaviors
- Ablation of LH GABAergic cells attenuates weight gain, consumption, and motivation
- LH GABAergic neuronal subsets are molecularly distinct from MCH and Orx cells
- In vivo Ca²⁺ imaging reveals separate appetitive and consummatory encoding networks

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In Brief
Deep brain calcium imaging in freely behaving mice reveals that appetitive and consummatory behaviors are encoded in distinct neurons in the hypothalamus, suggesting the existence of separate networks regulating motivation to eat and food consumption.
Visualizing Hypothalamic Network Dynamics for Appetitive and Consummatory Behaviors


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http://dx.doi.org/10.1016/j.cell.2014.12.026

SUMMARY

Optimally orchestrating complex behavioral states, such as the pursuit and consumption of food, is critical for an organism’s survival. The lateral hypothalamus (LH) is a neuroanatomical region essential for appetitive and consummatory behaviors, but whether individual neurons within the LH differentially contribute to these interconnected processes is unknown. Here, we show that selective optogenetic stimulation of a molecularly defined subset of LH GABAergic (Vgat-expressing) neurons enhances both appetitive and consummatory behaviors, whereas genetic ablation of these neurons reduced these phenotypes. Furthermore, this targeted LH subpopulation is distinct from cells containing the feeding-related neuropeptides, melanin-concentrating hormone (MCH), and orexin (Orx). Employing in vivo calcium imaging in freely behaving mice to record activity dynamics from hundreds of cells, we identified individual LH GABAergic neurons that preferentially encode aspects of either appetitive or consummatory behaviors, but rarely both. These tightly regulated, yet highly intertwined, behavioral processes are thus dissociable at the cellular level.

INTRODUCTION

Complex, but evolutionary well-conserved, behavioral states, such as those related to feeding, require both precurrent and consummatory responses (Sherrington, 1906). These oftentimes sequential reactions, which are historically conceptualized as appetitive and consummatory behaviors (Ball and Balthazart, 2008; Craig, 1917; Lorenz, 1950; Tinbergen, 1951), represent highly intertwined processes that are not fully distinguished at the neural level. The lateral hypothalamus (LH), a critical modulator of both appetitive and consummatory processes, is a heterogeneous brain area containing numerous genetically distinct cell populations that utilize various signaling modalities (Berthoud and Münzberg, 2011). Gene expression patterns within the LH suggest that individual neurons likely release either inhibitory or excitatory neurotransmitters, γ-aminobutyric acid (GABA), and glutamate, as well as a host of neuropeptides (Hökfelt et al., 2000; Lein et al., 2007), implying that identifiable subdivisions within the global LH neuronal network can be genetically targeted. Electrical stimulation of the LH, which nonspecifically activates various cell types and processes, evokes voracious consummatory feeding, as well as appetitive reward-related behaviors (Hoebel and Teitelbaum, 1962; Margules and Olds, 1962; Olds and Milner, 1954; Wise, 1971), whereas ablation of the region results in emaciation and aphagia (Anand and Brobeck, 1951; Hoebel, 1965). Moreover, the activity of LH neurons changes in response to food and associated stimuli (Ono et al., 1981, 1986). These electrically evoked behaviors and feeding-specific activity patterns were discovered in numerous species of the Kingdom Animalia, from lizards (Molina-Borja and Gómez-Soutullo, 1989) to humans (Quaade et al., 1974). These past findings emphasize the importance and evolutionary conservation of the LH for regulating these survival-oriented behaviors. However, given the heterogeneous cellular composition of the LH (Adamantidis and de Lecea, 2009; Karnani et al., 2013; Knight et al., 2012), and the fact that multiple fibers of passage traverse this region (Hahn and Swanson, 2012), classical electrical stimulation, lesion, or electrophysiological recording studies are unable to determine whether genetically defined cell types, such as LH GABAergic neurons, encode, and regulate precise aspects of appetitive food-seeking and/or consummatory behaviors.

Neural circuit tracing and manipulation experiments revealed that optogenetic modulation of LH glutamatergic neurons influences feeding and motivated behavioral responding (Jennings et al., 2013a). In the current study, we examined if molecularly defined LH neurons that express the gene for the vesicular GABA transporter (Vgat), and thus synthesize and release...
GABA, selectively promote and encode appetitive and consummatory behaviors.

RESULTS

Optogenetic Stimulation and Inhibition of LH GABAergic Neurons Bidirectionally Modulate Feeding and Reward-Seeking Behavior

Given that electrical stimulation of the LH produces multiple behavioral responses, including feeding and positive reinforcement (Hoebel and Teitelbaum, 1962; Margules and Olds, 1962), and that the highest concentration of GABA in the hypothalamus is located within the anterior portions of the LH (Kimura and Kuriyama, 1975), we first explored the functional role of LH GABAergic neurons in modulating feeding and reward-related behaviors by using optogenetic techniques. Applying established viral procedures (Jennings et al., 2013b), we first expressed channelrhodopsin-2 conjugated to enhanced yellow fluorescent protein (AAV5-ER1α-DIO-hChR2(H134R)-eYFP) selectively in GABAergic neurons located within the anterior and dorsolateral subdivisions of the LH (Figures 1A and 1B) in Vgat-ires-Cre mice (Vong et al., 2011) and implanted optical fibers directly above the LH for somata photostimulation (Figure 1C; Figure S1A available online). Approximately 4 weeks after surgery, we tested whether direct photostimulation of LH GABAergic neurons influenced feeding and reward-related behavioral phenotypes in ad-libitum-fed mice. Photoactivation of these neurons at 20 Hz significantly increased the time spent in a designated food area (Figures 1D and 1E), food consumption (Figure 1F), time spent in a location paired with photostimulation (Figure 1G), and optical self-stimulation behavior (Figure 1H).

Next, we tested whether photoinhibition of LH GABAergic neurons disrupted feeding and reward-related behaviors in food-restricted mice. Utilizing similar procedures as described above, we targeted a modified variant (Mattis et al., 2012) of the inhibitory opsin, archaerhodopsin-3 (AAV5-ER1α-DIO-eArch3.0-eYFP; Chow et al., 2010), to LH GABAergic neurons in Vgat-ires-Cre mice (Figures 1I–1K; Figure S1B). Photoinhibition of LH GABAergic neurons led to significant reductions in time spent in a designated food area (Figures 1L and 1M), food consumption (Figure 1N), and time spent in a location paired with photoinhibition (Figure 1O). These data indicate that selective optogenetic modulation of neurochemically distinct LH GABAergic neurons (Figures S1C–S1Q) influences both feeding and reward-related phenotypes, suggesting that both appetitive and consummatory behavioral processes are represented in the LH by GABAergic neurons.

Chemogenetic Activation of LH GABAergic Neurons Enhances Consummatory Behaviors

To expand upon the acute behavioral effects observed from optogenetic manipulations (Figure 1), we investigated if sustained activation of LH GABAergic neurons, over a longer timescale, influenced consumption and work effort to earn a caloric reward. Thus, we virally targeted the Gq-coupled excitatory designer receptor exclusively activated by designer drugs (DREADD), hM3Dq, to LH GABAergic neurons by injecting the Cre-inducible viral construct, AAV8-hSyn-DIO-hM3D(Gq)-mCherry (Krashes et al., 2011), into similar target zones within the LH of Vgat-ires-Cre mice (LH GABA::hM3Dq; Figures 2A and 2B; Figures S2A–S2G). The inert molecule, clozapine-N-oxide (CNO), selectively binds to hM3Dq and activates neurons through Gq signaling pathways (Alexander et al., 2009). Therefore, to verify CNO-mediated activation in LH GABA::hM3Dq neurons, we performed whole-cell recordings in brain slices and found that the spontaneous firing rate of a subset of LH GABA::hM3Dq neurons significantly increased upon CNO (5 μM) bath application (Figures 2C and 2D). Additionally, we examined whether in vivo stimulation of LH GABAergic neurons, via systemic CNO administration, enhanced the expression of Fos, a marker for neuronal activity, within the LH. Intraperitoneal (i.p.) injections of CNO (1 mg/kg) in LH GABA::hM3Dq mice significantly increased Fos expression in the LH compared to LH GABA::Control mice injected with CNO (Figures 2E–2I). To determine whether DREADD-mediated activation of LH GABAergic neurons affected consummatory behavior, mice were trained in a free-access caloric consumption task (1-hr duration) that permitted the quantification of lick responses at the delivery spout for a palatable caloric liquid reward. Chemogenetic activation of LH GABAergic neurons in LH GABA::hM3Dq mice via CNO (1 mg/kg, i.p.) injection led to a significant increase in the number of lick (consummatory) responses when compared to controls (Figures 2J and 2K). In addition, CNO administration 45 min prior to a 1 hr free-access feeding task (Figure 2L) enhanced food intake in ad-libitum-fed LH GABA::hM3Dq mice (Figure 2M; see the Extended Experimental Procedures).

To examine whether chemogenetic activation of LH GABAergic neurons alters work effort exerted to earn a caloric reward, we initially trained mice on a fixed ratio of one (FR1) schedule, where each active nose poke resulted in the delivery of a palatable and calorie-dense liquid. Following stable behavioral responding, on subsequent sessions, LH GABA::hM3Dq and LH GABA::Control mice were either administered saline or CNO (counterbalanced) 45 min prior to progressive ratio 3 (PR3) test sessions, which is an established behavioral assay for measuring an animal’s motivation to obtain caloric rewards (Krashes et al., 2011). Intriguingly, activation of these cells significantly increased lick responses (metrics of consumption; Figures 2N and 2O), but did not alter the number of active nose pokes nor the break point (metrics of motivation; Figures 2P and 2Q). Anecdotally, we observed that LH GABA::hM3Dq mice treated with CNO spent majority of the time licking at the reward receptacle (despite reward delivery being contingent upon nose poke responding), suggesting that bulk chemogenetic activation of these neurons may override optimal behavioral performance by biasing behavior toward aspects of consumption. Taken together, these data indicate that chemogenetic activation of LH GABAergic neurons enhances consummatory behaviors.

Vgat-Targeted LH GABAergic Neurons Are Molecularly Distinct from MCH and Orx Cells

The LH exclusively houses two separate molecularly defined cell types, melanin-concentrating hormone (MCH) and orexin (Orx) neurons (Elias et al., 1998), which are known to have diverse roles in regulating food intake, energy balance, reward, and...
sleep-wakefulness (Jego et al., 2013; Karnani et al., 2011; Sakurai et al., 1998; Whiddon and Palmiter, 2013). Because MCH and Orx are thought to promote aspects of feeding and reward-related behaviors, we next assessed whether V{	extsuperscript{G}a}t{	extsuperscript{-}targeted neurons within the LH coexpress either of these orexigenic neuropeptides. To achieve this, we immunolabeled both peptide-producing neuronal populations in V{	extsuperscript{G}a}t-IRES-Cre mice that endogenously express eYFP in V{	extsuperscript{G}a}t{	extsuperscript{+}} cells (V{	extsuperscript{G}a}t-eYFP) and determined whether Orx and/or MCH neurons colocalize with V{	extsuperscript{G}a}t{	extsuperscript{+}} cells in the LH. Strikingly, immunostaining for MCH and Orx in V{	extsuperscript{G}a}t-eYFP (V{	extsuperscript{G}a}t-IRES-Cre mice crossed to an Ai3 reporter line; Madisen et al., 2010) brain slices revealed that V{	extsuperscript{G}a}t{	extsuperscript{+}} LH neurons do not coexpress either of these neuropeptides, signifying that these V{	extsuperscript{G}a}t-targeted neurons in the LH represent a neurochemically distinct GABAergic subpopulation that is separate from MCH and Orx cells (Figures 3A–3F; Figure S3).
Figure 2. Bulk Chemogenetic Activation of LH GABAergic Neurons Enhances Consummatory Behaviors

(A) Viral targeting of AAV8-hSyn-DIO-hM3D(Gq)-mCherry into the LH of Vgat-IRES-Cre mice.

(B) 20x confocal image showing hM3Dq-mCherry expression in the LH of a Vgat-IRES-Cre animal. Scale bar, 200 μm.

(C) Example current-clamp traces from a LHGABA::hM3Dq brain slice before (baseline) and after 5 μM CNO demonstrating DREADD-mediated action potentials (bottom).

(D) CNO significantly increases spontaneous firing of LHGABA::hM3Dq neurons (n = 3 cells; n = 3 mice; t4 = 12.370; p = 0.0002).

(E) 20x confocal image from an example LHGABA::hM3Dq animal, where Fos-induction was assessed 2 hr after CNO (1 mg/kg; i.p.) injection. Scale bar, 500 μm.

(F–H) 63x confocal images displaying Fos immunoreactivity (F) and hM3Dq-mCherry expression (G) in a LHGABA::hM3Dq animal injected with CNO. (H) Merged image of (F) and (G) showing colocalization of Fos and hM3Dq-mCherry expression as indicated by white arrows. Scale bars, 20 μm.

(I) CNO administration in LHGABA::hM3Dq mice significantly increases Fos expression in the LH compared to controls and neighboring regions (n = 3 LH sections; n = 3 mice per group; F1,8 = 46.00; p < 0.0001).

(J) Cumulative lick responses from individual LHGABA::hM3Dq and LHGABA::Control mice during a single 1 hr free-access caloric consumption task.

(K) Systemic CNO application significantly increases lick responses in LHGABA::hM3Dq mice compared to LHGABA::Control mice and saline injections during a free-access caloric consumption task (n = 6 mice per group; F1,20 = 8.12; p = 0.01).

(L) Example color maps from LHGABA::hM3Dq (top) and LHGABA::Control mice (bottom) during the free-access feeding task.

(M) Systemic application of CNO in LHGABA::hM3Dq mice significantly increased food consumption during the free-access feeding task when compared to controls and saline injections (n = 6 mice per group; F1,20 = 6.37; p = 0.02).

(N) Cumulative lick responses from example LHGABA::hM3Dq and LHGABA::Control animals during a single 1 hr progressive ratio 3 (PR3) session.

(legend continued on next page)
Although these neuropeptide-producing neurons have been implicated as crucial modulators of feeding, selective activation of Vgat-targeted neurons within the LH does not directly stimulate MCH and Orx cells to produce feeding and reward-related behaviors (Figures S1F–S1Q and S2B–S2G).

Genetic Ablation of LH GABAergic Neurons Reduces Consummatory Behaviors and Motivation to Obtain Caloric Rewards
Next, we assessed the necessity of LH GABAergic neurons for regulating these feeding-related processes with cell-type-specific ablation methods. To selectively ablate LH GABAergic neurons, we injected a Cre-inducible viral construct coding for taCasp3-2A-TEVp (AAV2-FLEX-taCasp3-TEVp) into the LH of Vgat-IR-E-4Cre mice (LHGABA::taCasp3; Figure 3G) (Yang et al., 2013). In situ hybridization for glutamic acid decarboxylase (GAD67), a marker for GABAergic neurons (Erlander et al., 1991), revealed a significant reduction in the number of LH GABAergic neurons following bilateral LH injections of AAV-FLEX-taCasp3-TEVp, and no change in the number of GABAergic neurons within neighboring regions, including the entopeduncular nucleus (EP) and ventromedial hypothalamus (VMH; Figures 3H–3J). Previous studies demonstrated that chemical or electrolytic lesions of the LH can result in significant aphagia and weight loss (Harrell et al., 1975; Schallert and Whishaw, 1978). Therefore, we monitored the daily body weight of LHGABA::taCasp3 and LHGABA::Control mice, while both groups were maintained on a calorie-dense diet for 60 days. Ablation of LH GABAergic neurons significantly blunted weight gain in LHGABA::taCasp3 mice compared to controls (Figure 3K). Additionally, LHGABA::taCasp3 mice displayed a significant reduction in daily food intake measured at 1 month postvirus injection (Figure 3L).

Next, we tested the effects of LH GABAergic neuron ablation on acute feeding and motivation to obtain caloric reward. Food-restricted LHGABA::taCasp3 mice showed reduced food intake in the acute free-access feeding assay (Figures 3M and 3N) and diminished lick responding in the free-access caloric consumption task compared to controls (Figure 3O). When tested on the PR3 task, LHGABA::taCasp3 mice exhibited reduced lick responding compared to controls (Figures 3P and 3Q). Congruently, LHGABA::taCasp3 mice showed a significant reduction in metrics of appetitive responding (active nose pokes and break points) compared to controls (Figures 3R–3T). Furthermore, genetic ablation of LH GABAergic neurons did not significantly alter the number of MCH or Orx cells in the LH (Figures S4A–S4F), corroborating our findings that Vgat-targeted LH neurons are a separate population from MCH and Orx cells (Figures 3A–3F; Figure S3). Lastly, LHGABA::taCasp3 mice did not display locomotor deficits or anxiety-related phenotypes when tested in an open-field test (Figures S4G and S4H). Together, these data highlight a causal and specific role for LH GABAergic neurons in regulating appetitive responding, consumption, and energy balance.

In Vivo Ca²⁺ Imaging in Large Populations of LH GABAergic Neurons
The optogenetic, chemogenetic, and cell ablation experiments outlined above demonstrate that bulk modulation of LH GABAergic neurons causally regulates appetitive and consummatory behavioral output. Though these approaches provide important causal information for linking cell-type-specific function to behavior, bulk modulation of even genetically defined neurons fails to account for the high degree of functional diversity within a targeted cell population, and they do not accurately reflect endogenous cellular activity dynamics that underlie complex behaviors. Therefore, it is unclear whether appetitive and consummatory processes are orchestrated by functionally discrete LH GABAergic subpopulations or whether individual neurons are participant in aspects of both appetitive and consummatory behaviors. To examine this in detail, we applied in vivo microendoscopic imaging strategies (Barretto et al., 2011; Flusberg et al., 2008; Ghosh et al., 2011; Jennings and Stuber, 2014) to resolve somatic Ca²⁺ activity dynamics from hundreds of LH GABAergic neurons (n = 743 cells) in freely behaving mice (n = 6 mice; Movie S1). First, we expressed the green fluorescent sensor of neuronal activity, GCaMP6m, in LH GABAergic neurons by injecting a Cre-inducible AAV viral construct (AAVDJ-EF1a-DIO-GCaMP6m) into the LH of Vgat-IR-ES-Cre mice (LHGABA::GCaMP6m; Figures 4A–4C; Figures S5A–S5L). To circumvent the optical aberrations and the turbidity of tissue that typically preclude imaging in deep brain structures like the LH (~5 mm deep), we implanted 8-mm-long microendoscopes (0.5 mm diameter; consisting of a relay lens fused to doublet gradient refractive index microlenses) directly above the LH for optical detection of GCaMP6m fluorescence emission (Figures 4D–4F; Figure S5M). Next, we interfacd the implanted microendoscope with a detachable miniaturized fluorescence microscope (Ghosh et al., 2011; Ziv et al., 2013) to visualize Ca²⁺ signals from large populations of LH GABAergic neurons in freely moving LHGABA::GCaMP6m mice (Figure 4G). Furthermore, applying this technique in conjunction with established computational algorithms (Mukamel et al., 2009), we were able to identify and track the Ca²⁺ activity from individual LH GABAergic neurons within single recording sessions and across multiple days and behavioral tasks (Figures 4H and 4I; see the Extended Experimental Procedures).

Food-Location Coding Profiles of LH GABAergic Neurons
Because the activity of unclassified LH neurons can be modulated in response to nutrient-related information (Ono et al., 1981, 1986), we first determined whether LH GABAergic ensembles are engaged when food is readily available. Thus, we...
Figure 3. Genetic Ablation of LH Vgat Neurons that Are Separate from MCH and Orx Cells Attenuates Weight Gain, Food Seeking, and Consummatory Behaviors

(A–C) 20x confocal images of the dorsolateral LH demonstrating the absence of colocalization between Vgat-eYFP and MCH immunostaining (n = 223 ± 13.77 Vgat-eYFP cells per mm², n = 76 ± 11.37 MCH cells per mm², and 0% overlap; n = 3 LH sections; n = 3 Vgat-eYFP mice). Scale bars, 100 μm.

(D–F) Representative 20x confocal images from a Vgat-eYFP brain slice (D) immunostained for Orx in red (E) displaying a lack of eYFP and Orx-immunoreactivity coexpression in Vgat⁺ LH cells (F); n = 266 ± 24.2 Vgat-eYFP cells per mm², n = 200 ± 4.41 Orx cells per mm², and 0% overlap; n = 3 LH sections; n = 3 Vgat-eYFP mice). Scale bars, 100 μm.

(G) Viral injection of AAV2-FLEX-taCasp3-TEVp into the LH of Vgat-ires-Cre mice.

(H and I) 20x confocal images demonstrating decreased GAD67 expression in LHGABA::taCasp3 mice (H) compared to LHGABA::Control animals (I). Scale bars, 200 μm.

(J) GAD67 expression is significantly decreased in the LH of LHGABA::taCasp3 animals compared to LHGABA::Controls. Ablation of LH GABAergic neurons does not significantly alter GAD67 expression levels within the VMH and EP of LHGABA::taCasp3 and LHGABA::Control mice (n = 3 LH sections; n = 3 mice per group; F2,15 = 5.58; p = 0.01).

(K) Ablation of LH GABAergic neurons significantly blunted weight gain induced from a calorie-dense diet (n = 7 mice per group; F1,720 = 377.01; p = 0.0017).

(L) Four weeks after taCasp3-TEVp viral injection, LH GABAergic cell death significantly reduced daily consumption of a calorie-dense diet (n = 7 mice per group; t12 = 2.597; p = 0.0234).

(M) Color map locations from example LHGABA::taCasp3 (top) and LHGABA::Control (bottom) mice during the free-access feeding paradigm.

(N) LHGABA::taCasp3 mice display significant decreases in food consumption when compared to controls during the free-access feeding paradigm (n = 7 mice per group; t12 = 3.293; p = 0.0071).

(O) LH GABAergic neuron ablation significantly decreases lick responses in LHGABA::taCasp3 animals compared to controls during the PR3 task (n = 6 mice per group; t10 = 5.3320; p = 0.0002).

(P) Lick responses from example LHGABA::taCasp3 and LHGABA::Control animals during a single (1 hr) PR3 session.

(Q) Ablation of LH GABAergic neurons significantly decreases lick responses in LHGABA::taCasp3 animals compared to LHGABA::Controls during the PR3 task (n = 6 mice per group; t10 = 3.024; p = 0.012).

(R) Nose poke responses from example LHGABA::taCasp3 and LHGABA::Control animals during a single PR3 session.

(S) Ablation of LH GABAergic neurons significantly decreases nose poke responding in LHGABA::taCasp3 animals compared to LHGABA::Controls during the PR3 task (n = 6 mice per group; t10 = 2.773; p = 0.019).

(T) LHGABA::taCasp3 mice display significantly lower break points when compared to LHGABA::Controls during the PR3 session (n = 6 mice per group; t10 = 2.692; p = 0.022).

All values are mean ± SEM. Student’s t test or two-way ANOVA. See also Figures S3 and S4.
characterized the response profiles of individual LH GABAergic neurons during the same free-access feeding task used above and recorded Ca\(^{2+}\) signals as food-restricted LH\(^{GABA::}\)GCaMP6m mice freely explored an arena that possessed discrete food-containing zones (FZ) in two of the outer corners (Figure 5A; Movie S2). This approach allowed for a visual representation of discrete spatial Ca\(^{2+}\) responses (Figures 5B and 5C) with some neurons exhibiting increased Ca\(^{2+}\) spiking in the presence of food (Figures 5B and 5D) and others showing decreased Ca\(^{2+}\) transients while the animal was in the FZ (Figures 5C and 5E). Further, we calculated a response ratio for each LH GABAergic neuron based on the frequency of Ca\(^{2+}\) responses in the FZ over the frequency in nonfood zones (FZ to NFZ; Figure 5F). We categorized response profiles from individual neurons as food zone excited (FZe) or food zone inhibited (FZi) if their log FZ/NFZ ratios were > ±1 SD of the mean (0.0 ± 0.3) of the entire population (n = 612 cells). Total Ca\(^{2+}\) activity from both groups decreased over time (Figure S6); however, average responses to FZ and NFZ areas within FZe and FZi neurons revealed significant differences in their respective directions (Figures 5G and 5H), supporting the design of our classification model. To spatially represent the response profiles of each neuron, we pseudocolored individual cells from an example animal’s cell map by its log-transformed response ratio and observed that cells of differing response profiles intermingled with each other rather than segregating into separate clusters (Figure 5I). These data demonstrate that the endogenous activity dynamics of subsets of LH GABAergic neurons are preferentially modulated in environmental locations paired with food.

Individual LH GABAergic Neurons Selectively Encode Aspects of Appetitive or Consummatory Behaviors

Next, we sought to determine whether individual LH GABAergic neurons selectively compute aspects of appetitive and/or consummatory behaviors. Therefore, we tracked Ca\(^{2+}\) dynamics in LH GABAergic neurons during the same PR3 task described above, where LH\(^{GABA::}\)GCaMP6m mice worked to obtain a caloric liquid reward. Numerous individual LH GABAergic neurons showed time-locked Ca\(^{2+}\) transients in response to either the first lick after reward delivery (consummatory responses) or unreinforced active nose pokes (appetitive responses; Figures 6A and 6B; Movie S3). Nonconsummatory licks, those following unreinforced nose pokes, also evoked Ca\(^{2+}\) changes but to a much lesser degree (Figure S7A),
signifying that these consummatory Ca\(^{2+}\) responses depend on the presence of the caloric reward. We profiled all imaged neurons based on their differences between the average Ca\(^{2+}\) activity 1.5 s before and after each behavioral event to quantify their responses to aspects of feeding. LH GABAergic neurons displayed a variety of classifiable response profiles, such as cells that were modulated during reward consumption or immediately following active nose pokes (Figures 6C and 6D; Figures S7B–S7E). Thus, we categorized these neurons as responsive to a particular behavioral event (consumption or nose poke) if their Ca\(^{2+}\) activity (averaged from 0 to 1.5 s after the event) was statistically enhanced compared to the activity –1.5 to 0 s before the event. Averaging responses to consummatory licks revealed separate subpopulations of neurons with distinct Ca\(^{2+}\) responses (Figure 6E). A much larger population of LH GABAergic neurons displayed significant Ca\(^{2+}\) signals time-locked to appetitive nose pokes (Figure 6F), although the amplitudes of these responses were lower compared to consummatory responses. LH GABAergic neurons displayed a diverse range of responses to consummatory licks and nose pokes (Figures S7F and S7G). However, we found that individual neurons that significantly responded to reward consumption were largely separate from those that respond to appetitive nose pokes (Figures 6G and 6H). Taken together, these data demonstrate that functionally segregated subpopulations of LH GABAergic neurons encode aspects of appetitive (nose-poke-responsive cells) or consummatory (lick-responsive cells) behaviors, but rarely both.

Lastly, we explored the Ca\(^{2+}\) dynamics of the same neurons between both behavioral paradigms (free-access feeding and the PR3 task) to determine if the same neurons that respond to the presence of food during the free-access feeding task also...
responded to consummatory licks and/or nose pokes during the PR3 task. To accomplish this, we registered IC unit cell maps from the same animal between sessions (Figures 7A–7C) and applied a 5 μm cutoff between center points of paired cells based on the distribution of cells from different imaging session (Figure 7D) and the distance between cells from the same sessions (Figure 7E). Using these criteria, only a subset of neurons imaged during the free-access feeding behavioral task were also detected in the PR3 task (n = 40 PR3 excited cells out of 125 FZ-responsive cells from paired sessions. Total paired cells between recording sessions: 472; Figure 7F). Neurons tracked in both sessions were not localized to any portion of the field of view and showed no distinct anatomical pattern or layout (Figures 7G–7I). However, paired cells displayed a high degree of functional overlap in the schematized IC unit cell maps between distinct imaging sessions. A large proportion of FZe neurons that also respond to PR3 behavioral events (28/40 cells) displayed increased activity to either nose pokes or consummatory licks in the PR3 task, whereas a smaller portion of FZi neurons (12/40 cells) responded to PR3 behavioral events (Figure 7J). Taken together, these data reveal that subsets of LH GABAergic neurons functionally overlap between tasks that require different behavioral processes to obtain food, signifying the flexibility and complexity of these neurons for integrating and regulating components of feeding.

**DISCUSSION**

Historically, the LH has been viewed as a critical governor of both appetitive and consummatory behaviors (Hoebel and Teitelbaum, 1962; Margules and Olds, 1962). However, given that the LH encompasses a plethora of genetically, anatomically,
and functionally distinct neurons that utilize various neurotransmitters and neuropeptides, the precise mechanisms by which these cell populations orchestrate behavior have remained a mystery. The results described here demonstrate that Vgat-expressing neurons in the LH function to promote both appetitive and consummatory behaviors. Importantly, we show that these Vgat-targeted cells in the LH do not colocalize with either of the neuropeptides, MCH, or Orx, in agreement with previous findings that certain LH GABAergic subpopulations (GAD65-expressing neurons) are neurochemically and electrophysiologically distinct from MCH and Orx cells (Karnani et al., 2013). However, it has also been reported that some MCH neurons do express GABAergic markers, such as GAD67, and release GABA from distal synaptic terminals (Jego et al., 2013). Thus, transgene penetrance in Vgat-IRES-Cre mice may be reduced compared to endogenous LH Vgat expression and/or MCH neurons may express extremely low levels of Vgat, which can be dynamically regulated as observed in other GABAergic neuronal populations (Jarvie and Hentges, 2012; Lamas et al., 2001; Sperk et al., 2003). In addition, the present results do not rule out the possibility that these unique Vgat-targeted GABAergic neurons contain other neuropeptides, such as Neurotensin or Galanin (Allen and Cechetto, 1995; Goforth et al., 2014; Laque et al., 2013). Thus, parceling out precise neuronal subtypes from these multidimensional populations in the LH to then elucidate their function still remains a major challenge.

Divergent circuit connectivity between LH neurons and upstream and downstream circuit nodes could also account for the diverse appetitive and consummatory response profiles of the LH GABAergic neuronal population. Previous retrograde and anterograde tracing studies demonstrate strong connectivity between the LH and other feeding- and reward-associated brain regions that are also important for motivated behaviors, including other hypothalamic nuclei, midbrain, hindbrain, and striatal structures (Adamantidis et al., 2011; Betley et al., 2013; Gutierrez et al., 2011; Hahn and Swanson, 2012; Kempadoo et al., 2013; Leinninger et al., 2011), implying that the selective-coding properties within separate LH GABAergic subpopulations might be input and/or projection dependent.

While the LH controls both appetitive and consummatory behaviors, these processes are encoded by distinct cellular LH GABAergic subpopulations. Our data suggest that separate subsets of appetitive-coding and consumption-coding ensembles exist within the LH GABAergic network. Thus, the LH GABAergic network can be viewed as a mosaic of functionally and computationally distinct cell types, requiring further definition. Nevertheless, these important computational differences among individual LH GABAergic neurons would have gone unnoticed if only bulk neuromodulatory approaches were employed, further underscoring the necessity of identifying the natural activity dynamics within a network to better understand the precise neural underpinnings of complex behavioral states.
EXPANSION PROCEEDURES

Experimental Subjects
All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted by the NIH, and with approval of the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill (UNC). Adult (25–30 g) male Vgat-ires-Cre (Vong et al., 2011) or wild-type littermate mice were used.

Behavioral Assays
All food-deprived mice were restricted to 85% to 90% of their initial body weight by administering one daily feeding of ~2.5 to 3.0 g of standard grain-based chow immediately following each behavioral experiment, if performed. Animals were run on free-access feeding, real-time place preference, optical self-stimulation, and operant feeding (FR1 and PR3) assays. For optogenetic manipulations, light from solid-state lasers (473 or 532 nm) was delivered via custom-made patch cables to implanted chronic fibers on animals’ head as described previously (Jennings et al., 2013b). For each chemogenetic behavioral manipulation, mice received i.p. injections of either vehicle or CNO (1 mg/kg) 45 min prior to testing with at least 3 days in between each session (counterbalanced). For further details, refer to the Extended Experimental Procedures.

FREELY MOVING CA2+ IMAGING
A miniature microscope with an integrated LED (475 nm) was used to image GCaMP6m fluorescence in LH GABAergic neurons through an implanted microendoscope. Using nVista HD Acquisition Software (v. 2; Inscopix), images were acquired at 15 frames per second with the LED transmitting 0.1 to 0.2 mW of light on average. Ca2+ imaging was synchronized with time-stamped behavioral data at the start of each session. All images were processed using the Mosaic software (v. 1.0.0b; Inscopix) and then analyzed with custom MATLAB scripts. For further details, refer to the Extended Experimental Procedures.

Statistical Analysis
Mean values are accompanied by SEM values. Comparisons were tested using paired or unpaired t tests. Two-way ANOVA tests followed by Bonferroni post hoc comparisons were applied for comparisons with more than two groups.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Extended Experimental Procedures, seven figures, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2014.12.026.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS
We thank Spencer Smith, Ilana Witten, Mark Schnitzer, and the members of the Stuber lab for helpful discussion. We thank the UNC vector core for viral packaging, Bradford Lowell and Linh Vong for the Vgat-Ires-Cre mice, and the UNC Neuroscience Center Microscopy Core (P30 NS045892). This work is dedicated to Johnathon Gregory Taylor (1996–2014), whose contributions were greatly appreciated and valued for this project. This study was supported by The Klarman Family Foundation, The Brain and Behavior Research Foundation, the Foundation for Prader-Willi Research, the Foundation of Hope, the National Institute on Drug Abuse (DA032750 and DA038168), and the National Institute on Alcohol Abuse and Alcoholism (P60 AA011605). J.H.J. was supported by a grant from the National Institute of Mental Health (MH104013), and A.M.S. was supported by grants from the National Institute on Drug Abuse (DA034472). S.L.R. was supported by T32HD040127. S.O. is an employee of Inscopix, Inc.

Received: July 31, 2014
Revised: October 28, 2014
Accepted: November 24, 2014
Published: January 29, 2015

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